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Research report

Activation of migration of endogenous stem cells by erythropoietin as potential rescue for neurodegenerative diseases

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ABSTRACT

Neurodegenerative disorders such as Alzheimer's disease (AD) are characterized by progressive cognitive dysfunction and memory loss. There is deposition of amyloid plaques in the brain and subsequent neuronal loss. Neuroinflammation plays a key role in the pathogenesis of AD. There is still no effective curative therapy for these patients. One promising strategy involves the stimulation of endogenous stem cells. This study investigated the therapeutic effect of erythropoietin (EPO) in neurogenesis, and proved its manipulation of the endogenous mesenchymal stem cells in model of lipopolysaccharide (LPS)-induced neuroinflammation.

Methods: Forty five adult male mice were divided equally into 3 groups: Group I (control), group II (LPS untreated group): mice were injected with single dose of lipopolysaccharide (LPS) 0.8 mg/kg intraperitoneally (ip) to induce neuroinflammation, group III (EPO treated group): in addition to (LPS) mice were further injected with EPO in dose of 40 μg/kg of body weight three times weekly for 5 consecutive weeks. Groups were tested for their locomotor activity and memory using open field test and Y-maze. Cerebral specimens were subjected to histological and morphometric studies. Glial fibrillary acidic protein (GFAP) and mesenchymal stem cell marker CD44 were assessed using immunostaining. Gene expression of brain derived neurotrophic factor (BDNF) was examined in brain tissue.

Results: LPS decreased locomotor activity and percentage of correct choices in Y-maze test.

Cerebral sections of LPS treated mice showed increased percentage area of dark nuclei and amyloid plaques. Multiple GFAP positive astrocytes were detected in affected cerebral sections. In addition, decrease BDNF gene expression was noted. On the other hand, EPO treated group, showed improvement in locomotor and cognitive function. Examination of the cerebral sections showed multiple neurons exhibiting less dark nuclei and less amyloid plaques in comparison to the untreated group. GFAP positive astrocytes were also reduced. Cerebral sections of the EPO treated group showed multiple branched and spindle CD44 positive cells inside and around blood vessels more than in LPS group. This immunostaining was negative in the control group. EPO administration increased BDNF gene expression.

Conclusion: This study proved that EPO provides excellent neuroprotective and neurotrophic effects in vivo model of LPS induced neuroinflammation. It enhances brain tissue regeneration via stimulation of endogenous mesenchymal stem cells proliferation and their migration to the site of inflammation. EPO also up regulates cerebral BDNF expression and production, which might contributes to EPO mediated neurogenesis. It also attenuates reactive gliosis thus reduces neuroinflammation.

These encouraging results obtained with the use of EPO proved that it may be a promising candidate for future clinical application and treatment of neurodegenerative diseases.

1. Introduction

Alzheimer's disease (AD) is the most common progressive chronic neurodegenerative disorder in elder people and one of

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the leading causes of dementia. Memory loss is one of its earliest symptoms, along with a gradual decline of intellectual abilities, personality and behavioral changes (Maccioni et al., 2009; Webster et al., 2006;Yu et al., 2009). There are three major neuropathological hallmarks that have been identified in the brain of AD patients: amyloid plaques, neurofibrillary tangles (NFTs), and astrogliosis (Yu et al., 2009; Tang, 2009).

Neuroinflammation plays a significant role in the heterogeneous pathogenesis of AD (Korolainen et al., 2005; McNaull et al., 2010). The role of microglia in AD inflammation has long been acknowledged (Birch, 2014). Substantial evidence now demonstrates that astrocyte-mediated inflammatory responses also influence AD pathology but its exact role still remains elusive (Phillips et al., 2014). Astrocytes, mainly forming the cytoskeleton of the CNS, produce and release proinflammatory molecules that may be critical for the generation of amyloid-β peptide (Aβ). (Avila-Muñoz and Arias, 2014). Activated astrocytes express GFAP, a principal intermediate filament in mature astrocytes of the CNS and a member of the cytoskeletal protein family (Korolainen et al., 2005). GFAP has been used as a marker in determining the stellate morphology of astrocytes, which is maintained by the phosphorylation carried out by specific serine/threonine kinases. (Harrison and Mobley, 1992; Eng and Ghirnikar, 1994; Brenner et al., 2001). Astrocytes, contributing to the neuroinflammatory process in AD, become an attractive target for future therapeutic approaches.

The hematopoietic growth factor, erythropoietin (EPO) has a tissue protective function that is independent of its action on erythropoiesis. The presence of EPO and its receptor in the neurovascular system has generated an immense amount of interest to target EPO and its downstream pathways for novel therapeutic strategies against neurodegenerative disorders (Byts and Sirén, 2009). EPO can protect neurons from oxidative stress, spinal cord ischemia, retinal disease, stroke, and demyelinating disease (Maiese et al., 2012). EPO also stimulates neural cell proliferation and prevents neuron apoptosis by promoting oxygen delivery to brain or by direct interaction with neural cells (Zhi-Yong et al., 2007).

Neurological diseases are most difficult to treat and cure reflecting limited neurogenesis in the human central nervous system (CNS). It has been reported that bone marrow-derived mesenchymal stem cells (BMSCs) preferentially migrate to the injured tissue but with limited efficiency. These mesenchymal stem cells can generate neurons, which is not that surprising as the neuron is the default program in stem cell differentiation. Activation of endogenous neuronal stem cells (NSCs) to repair damaged neurons in CNS may be the best strategy for treating AD because it can avoid ethical and biological issues of using the exogenous stem cells (Li et al., 2015b).

Therefore, the present work was designed to study whether the endogenous neurogenesis could be enhanced by EPO treatment in an animal model of LPS induced neuroinflammation, focusing on the possible effect of EPO on BMSC migration to the brain, and its impact on improving the pathology and cognitive decline symptoms associated with this model.

2. Materials and methods

2.1. Animals

Forty five adult male mice weighing between 25 and 30 g were used in this study. Animals were purchased from the animal house of the National Research Center (NRC, Giza, Egypt). All experimental procedures were conducted according to NIH (National Institute of Health) guidelines for the treatment and care of laboratory animals published in the NIH publication 85–23 revised 1985.

Animals were housed in separate plastic cages (fifteen per cage) in the laboratory animal center of the German University in Cairo, under controlled temperature (22 ± 2 °C) and 50 ~ 55% relative humidity under a 12-h light/12-h dark cycle, for one week for stabilization. All animals had free access to food (standard diet) and water. All experiments were performed during the daylight hours.

2.2. Chemical

The following chemicals were purchased from the respective suppliers as stated below:

1. Lipopolysaccharide (LPS) was used in this research to induce neuroinflammation (Kovács et al., 2014). It was purchased from (Sigma–Aldrich, USA) from LPS (*Escherichia coli*, serotype 0127:B8, Sigma, cat number: 63H-4010).
2. EPO (Epoetin 4000 IU/1ml of solution–SEDICO Pharmaceutical Co., 6 October City Cairo).
3. Rabbit polyclonal antibody for GFAP immunostaining catalog number (AB5804)
4. Primary antibody (CD44) rabbit polyclonal antibody, catalogue number (Immunohistochemistry world IW-PA1021)

All other reagents and chemicals were of the highest quality commercially available.

2.3. Experimental protocol

Animals were equally divided into three groups, each containing 15 mice. The first group, served as control, receiving a daily dose of 1% tween 80 used as a vehicle, injected intraperitoneally (i.p.) for 7 days.

In the present study, LPS from the cell wall of gram-negative bacteria was used to produce chronic, global inflammation within the brain of mice. LPS was injected to mice of group II and III in a single dose of 0.8 mg/kg i.p. This was followed by a daily dose of 1% tween 80 for 7 days (Arai et al., 2001).

One group was left without any treatment and served as the diseased group (group II). One week later, group III mice were treated by i.p., injection of EPO at a dose of 40 μg/kg of body weight three times weekly for 5 consecutive weeks (Arai et al., 2001; Sheng et al., 2003).

2.4. Measured parameters

2.4.1. Behavioral tests

Behavioral tests were performed for group II one week after LPS injection, while group III were tested after six weeks from the start of the experiment

2.4.1.1. The open field test. The open field test is a locomotor behavior assessment test (Samuel et al., 2008), which is used primarily to examine motor function by means of measuring spontaneous activity in an open field. The test was carried out in a big square metal box, each side of its base measures 80 cm in length and its height measures 40 cm (Holmes et al., 2003; Hefner et al., 2007). The box walls were painted red in color while the floor was left white and it was divided into 16 equal squares.

Mice were placed individually into the central point of the open field and observed during a 3 min period, the floor and walls were cleaned after testing each mouse. The following parameters were recorded:

1. Ambulation frequency: represents the number of squares crossed by each mouse per minute. Entries were recorded manu-